Remarks

Applicants have amended the claims to expedite prosecution of a preferred embodiment. Specifically, claims 1, 9, 12, and 17 have been amended to specify that the amplified fragment is about 100 bp long. Support for the amendment can be found, for example, in paragraphs [015] and [077]. Accordingly, claim 24 has been cancelled. Applicants have further amended claim 1 to make explicit that which was implicit, namely that in step (c), one genotypes also a third nucleic acid region. Applicants have also amended claims 4-6 as suggested by the Examiner. The amendments are clerical.

Accordingly, no new matter has been introduced by the amendments and their entry is respectfully requested.

Applicants now turn to the specific rejections.

The Examiner rejected claims 1-8 and 19-24 as allegedly not complying with 35 U.S.C. §112, second paragraph, definiteness requirement.

Specifically, the Examiner noted that claims 1-8 and 19-24 were unclear over the recitation of the phrase 'genotyping the polymorphic site in the at least the first nucleic acid region and the second nucleic acid region thereby resulting in at least a first, a second, and a third genotype.' Applicants appreciate the Examiner's advise on amending claim 1 and have proceeded accordingly.

The Examiner further noted that claims 4-6 were unclear over recitation of the phrase 'the polymorphic marker is a' as recited in each of claims 4-6. Claim 1, from which rejected claims 4-6 depend requires 'at least three polymorphic markers.' Applicants appreciate the Examiner's advise on amending the claims and have proceeded accordingly.

The Examiner also noted that claim 24 was unclear over recitation of the phrase 'the region flanked by the first, the second, and the third nucleic acid is about 100 bp long'. Applicants have cancelled claim 24 and therefore, the rejection has been rendered moot.

In view of amendments to the claims, Applicants respectfully submit that all the claims now comply with 35 U.S.C. §112, second paragraph, definiteness requirement and that the rejections should be withdrawn.

The Examiner rejected claims 1, 2, 4-6, 8, and 21, 22, 24 under 35 U.S.C. 103(a) as allegedly being unpatentable over Ruano et al (1990) ("Ruano") in view of Furlong et al (1993) ("Furlong").

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

The Examiner acknowledged that Ruano not describe producing 12-18 replica genotypes, polymorphic markers that are three or more, or four or more kilo base pairs apart, or flanked regions that are about 100 bp long. The Examiner contended that "such methods were well known in the art at the time the invention was made."

The specification teaches that the present method achieves an extremely high efficiency for haplotyping (par. [077]). Such efficiency had not been described nor suggested by any of the prior art methods. Further, based on the description of existing methods, a skilled artisan would not have expected the present method to work as efficiently as it does based upon using short DNA fragments.

Applicants respectfully submit that the specific amplification of short fragments for the efficient multiplex amplification and accurate and reproducible detection of the fragments had not been shown or recognized prior to the present invention.

The Examiner further contended that Furlong discloses amplification of fragments of about 100bp. However, the smallest fragment Furlong amplified was 149bp-159bp. The other regions were larger (266-256bp and 242-222bp, see page 1192, 1st col. under heading "PCR Primers"). Thus, one set of primers amplified fragments that were over 250 bp long, a second set amplified fragments that were between 242-222 bps and one set amplified fragments between 149 and 159bp long. None of the primers amplified a fragment that was about 100bp. Even if a skilled artisan would interpret the term "about" to include fragment sizes varying by 50%, an interpretation with which Applicants do not agree, only one fragment of Furlong comes close to that range. The claims require that all the at least three or more fragments that are amplified in the claimed multiplexing method, must be small to achieve the best efficiency.

As explained in the specification, particularly the description of the Figures and the examples, one of the problems that can occur is a failed multiplex assay. The failure of such an assay can be due to no template present or from failed PCR amplification. One type of failed

PCR amplification is partially failed genotyping where only 1 or 2 SNPs are successfully genotyped. This is most likely due to unsuccessful PCR for 1 or 2 of the amplified DNA regions. The haplotyping can be successfully achieved by improving the PCR efficiency. This is achieved by using **short sequences** around **each SNP** – about 100 bp. Using such short sequences **should be done around each SNP** – not only one or two. Otherwise, the advantages in getting all three or more SNP replicated are not achieved. This simply is not taught by the references. In fact, if anything, it is taught against.

Accordingly, in view of the amendments and the arguments set forth, *supra*, Applicants respectfully submit that the rejection should be withdrawn.

The Examiner rejected claim 7 under 35 U.S.C. 103(a) as allegedly being unpatentable over Ruano in view of Furlong et al (1993) and further in view of Ross et al (citations no. 27 on the IDS of 7/31/2006)("Ross").

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

As described, *supra*, the combination of Ruano and Furlong does not teach or suggest the elements of the amended claims. Specifically, the combination does not teach that all of the fragments amplified in a multiplex reaction should be about 100bp long or any reason for doing so. In fact, the combination of the references suggests that segments over 250 bps can be used. If anything is taught, the references suggest using fragments that fall in the mid to upper 200bp range.

Ross does not overcome this deficiency because it does not teach this missing element. All Ross describes is a method for genotyping using primer extension and mass spectrometry. There is nothing in Ross that would teach or suggest a skilled artisan to design a method of multiplex amplification for haplotyping from single molecule nucleic acid dilutions using only fragments that are about 100bp long.

In view of the amendments to claim 17 and the arguments set forth *supra*, Applicants respectfully submit that the rejection be withdrawn.

The Examiner rejected claims 3, 9-11 and 20 under 35 U.S.C. 103(a) as allegedly being unpatentable over Ruano in view of Furlong et al (1993) and further in view of Drysdale et al (2000) (citation no. 9 on the IDS of 07/31/2006)("Drysdale").

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

As described, *supra*, the combination of Ruano and Furlong does not teach all the elements of the amended claims. Specifically, the combination does not teach that all the fragments amplified in a multiplex reaction should be about 100bp long. Rather, it teaches the opposite.

Drysdale does not overcome this deficiency because it does not teach this missing element. All Drysdale describes is the use of (β_2AR) receptor haplotypes comprised of 13 polymorphic positions in the prediction of response to albuterol (p.10486, left col., Ins.6-8), which is a biological trait. There is nothing in Drysdale that would teach or suggest a skilled artisan to design a method of multiplex amplification for haplotyping from single molecule nucleic acid dilutions using only fragments that are about 100bp long.

In view of the claim amendments and the arguments set forth *supra*, Applicants respectfully submit that the rejection be withdrawn.

The Examiner rejected claims 12-18 under 35 U.S.C. 103(a) as allegedly being unpatentable over Ruano in view of Furlong and further in view of Rein et al (1998) (citation no. 26 on the IDS of 07/31/2006) ("Rein") and Buckholz et al (1997) (" Buckholz").

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

As described, *supra*, the combination of Ruano and Furlong does not teach all the elements of the amended claims. Specifically, the combination does not teach that all the fragments amplified in a multiplex reaction should be about 100bp long.

Neither Rein nor Buckholz, nor combination thereof, overcomes this deficiency because they do not teach this missing element. As indicated by the Examiner, all Rein describes is method for the identification of 5-metyhlcytosine and related modifications in DNA genomes. There is nothing in Rein that would teach or suggest a skilled artisan to design a method of multiplex amplification for haplotyping from single molecule nucleic acid dilutions using only fragments that are about 100bp long. Also, as indicated by the Examiner, Buckholz only teaches the analysis of several epigenetically modified methylation sites greater that are about one or more kilo base pairs apart in the analysis of genomic imprinting and Prader Willi Syndrome.

In view of the claim amendments and the arguments set forth *supra*, Applicants respectfully submit that the rejection be withdrawn.

The examiner rejected claim 23 under 35 U.S.C. 103(a) as allegedly being unpatentable over Ruano in view of Furlong and further in view of Gerhard et al (1984).

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

As described, *supra*, the combination of Ruano and Furlong does not teach all the elements of the amended claims. Specifically, the combination does not teach that all the fragments amplified in a multiplex reaction must be about 100bp long.

Gerhard does not overcome this deficiency because it does not teach this missing element. All Gerhard describes is that a B-globin haplotype including polymorphic positions that are 15-20 kilo base pairs apart. There is nothing in Gerhard that would teach or suggest a skilled artisan to design a method of multiplex amplification for haplotyping from single molecule nucleic acid dilutions using only fragments that are about 100bp long.

In view of the claim amendments and the arguments set forth *supra*, Applicants respectfully submit that the rejection be withdrawn.

In view of the foregoing, the Applicants respectfully submit that all claims are in condition for allowance. Early and favorable action is respectfully requested.

In the event that any additional fees are required, the PTO is authorized to charge our deposit account No. 50-0850.

Date: October 21, 2008

Respectfully submitted,

/Leena H. Karttunen/

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